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The controversy over H5N1 transmissibility research

An opportunity to define a practical response to a global threat

David S. Fedson[1,*] and Steven M. Opal[2,3]

[1]Sergy Haut, France; [2]Center for Biodefense and Emerging Pathogens; Department of Medicine; Memorial Hospital of Rhode Island; Pawtucket, RI USA; [3]Warren Alpert Medical School of Brown University; Providence, RI USA

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Since December 2011, influenza virologists and biosecurity experts have been engaged in a controversial debate over research on the transmissibility of H5N1 influenza viruses. Influenza virologists disagreed with the NSABB's recommendation not to publish experimental details of their findings, whereas biosecurity experts wanted the details to be withheld and future research restricted. The virologists initially declared a voluntary moratorium on their work, but later the NSABB allowed their articles to be published, and soon transmissibility research will resume. Throughout the debate, both sides have had understandable views, but both have overlooked the more important question of whether anything could be done if one of these experimentally derived viruses or a naturally occurring and highly virulent influenza virus should emerge and cause a global pandemic. This is a crucial question, because during the 2009 H1N1 influenza pandemic, more than 90% of the world's people had no access to timely supplies of affordable vaccines and antiviral agents. Observational studies suggest that inpatient statin treatment reduces mortality in patients with laboratory-confirmed seasonal influenza. Other immunomodulatory agents (glitazones, fibrates and AMPK agonists) improve survival in mice infected with influenza viruses. These agents are produced as inexpensive generics in developing countries. If they were shown to be effective, they could be used immediately to treat patients in any country with a basic health care system. For this reason alone, influenza virologists and biosecurity experts need to join with public health officials to develop an agenda for laboratory and clinical research on these agents. This is the only approach that could yield practical measures for a global response to the next influenza pandemic.

Introduction

In December 2011, the National Science Advisory Board for Biosecurity (NSABB) in the US recommended restricting publication of the experimental details of A/H5N1 influenza virus transmissibility research conducted by Ron Fouchier, Yoshi Kawaoka and their colleagues.[1,2] Fouchier had presented the results of his studies at a scientific meeting in September 2011 and his findings had received considerable attention among influenza virologists. However, following the announcement of the NSABB recommendation, there was widespread comment in major scientific journals and in the media, and the NSABB's decision quickly became controversial.[3]

*Correspondence to: David S. Fedson; Email: dfedson@wanadoo.fr
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H5N1 Transmissibility Research and the NSABB

In response to the NSABB decision, Fouchier and Kawaoka reluctantly agreed to a voluntary moratorium on publishing their findings and continuing their research.[4] They and many other virologists were concerned that science was being censored.[1,2,5-9] In contrast, the NSABB[10,11] and others regarded as biosecurity experts[12-15] worried that a highly transmissible H5N1 virus could be released accidentally or deliberately among human populations. In February 2012, the World Health Organization (WHO) convened an international technical consultation that included the principal scientists involved in this controversy.[16] One month later, the NSABB received reassuring new data from Fouchier and Kawaoka. Moreover, intelligence officials had concluded that H5N1 transmissibility research did not present a biosecurity threat. Accordingly, the NSABB revised its earlier decision and unanimously recommended full publication of Kawaoka's findings,[17] which were subsequently published.[18] There was less than complete agreement on whether to publish Fouchier's findings, but after extensive revision his manuscript too was published.[19] The US Government also issued revised recommendations on its oversight of "dual use research of concern"; i.e., research that is considered scientifically useful but could also be used deliberately or accidentally to cause harm.[20]

Influenza virologists believe that publication of their findings will have several benefits. For example, Kawaoka has said, "The amino acid changes identified here will help individuals conducting surveillance in regions with circulating H5N1 viruses … to recognize key residues that predict the pandemic potential of

isolates. Rapid responses in a potential pandemic situation are essential in order to generate appropriate vaccines and initiate other public health measures to control infection. Furthermore, our findings are of critical importance to those making public health and policy decisions."[18] However, many influenza scientists doubt this research will yield any practical benefits for influenza virus surveillance or for developing vaccines and antiviral agents, at least in the foreseeable future.[21,22]

The ability of influenza viruses to mutate and yield new viruses that might be more virulent or more easily transmitted was earlier demonstrated in vivo for the 2009 pandemic A (H1N1) (pH1N1) virus in mice[23] and ferrets.[24-26] These reports appeared before the H5N1 studies of Fouchier and Kawaoka came to NSABB and public attention. A more recent study has reported the in vitro evolution of two mutant H5N1 viruses, one that was transmissible by direct contact and another that was partially transmissible by droplets in ferrets.[27] Fouchier and Kawaoka found that only 3 to 5 mutations were required to generate respiratory transmissible H5N1 viruses. Other investigators using mathematical models have concluded, "the remaining mutations could evolve within a single mammalian host, making the possibility of a respiratory droplet–transmissible A/H5N1 virus evolving in nature a potentially serious threat."[28]

The H5N1 transmissibility research controversy is slowly moving toward resolution. Eventually, new rules for this and other types of "dual use research of concern" will be formulated. In the meantime, it is worth asking whether this controversy has something else to teach us.[29]

Adequate Global Supplies of Vaccines and Antiviral Agents Won't be Available for a Global Response to the Next Pandemic

The concerns expressed by influenza virologists and biosecurity experts about H5N1 transmissibility research are understandable. However, both groups have overlooked a far more important question: could an effective global response be mounted to confront a pandemic caused by a new highly transmissible and virulent influenza virus, regardless of whether it is a laboratory-generated H5N1 virus or (more likely) a naturally derived variant of the currently circulating H5N1 or seasonal influenza viruses? This question is critically important, for if a virus as virulent as the one that caused the pandemic in 1918 were to emerge today, it might kill 62 million people worldwide.[30]

The global response to the relatively mild H1N1 influenza pandemic in 2009 amply demonstrated that scientists, companies and public health officials working together lacked the capacity to rapidly develop,[31] produce[32] and distribute[33-35] affordable supplies of pandemic vaccines and antiviral agents in time to mitigate the pandemic's impact on more than 90% of the world's people. This is incontrovertible evidence that in the event of a new and more severe influenza pandemic, regardless of its provenance, it will be impossible to successfully implement an effective global public health response that targets only the virus.

Clinical and Epidemiologic Findings Suggest an Alternative Approach to a Pandemic

If vaccines and antiviral agents will be unavailable to most of the world's people when the next pandemic virus emerges, would it be possible to confront the pandemic using an alternative approach that targets the host response to the virus? A clue to the promise of this approach promise can be seen in the disparity in the case fatality rates of children and young adults in the 1918 influenza pandemic.[36] This pandemic caused exceptional mortality in young adults but not in children. Some scientists have ascribed the high mortality in young adults to secondary bacterial pneumonia,[37-39] but this explanation fails to account for the more frequent infection of children with the virus that killed young adults and the (almost certain) more frequent colonization of their nasopharyngeal passages with the same bacteria found in the lungs of young adults who died (**Fig. 1**).[36,40]

Influenza virologists recognize that children were not protected from infection, but "… for reasons that are as mysterious today as they were in 1918, they were able to cope with the disease much better than their adult counterparts."[41] Although these virologists have made extraordinary contributions to our understanding of the 1918, H5N1 and other influenza viruses, they have been unable to answer the question, "Why did young adults die." The more important question is "Why did children live?" The different case fatality rates in children and young adults in 1918 might have been due to characteristics specific to host responses of children and young adults that differentially affected their risks of dying.[36,40] Clinicians and epidemiologists have documented similar differences in the case fatality rates of children and adults in several other infectious and non-infectious conditions.[40] These differences might have arisen during the course of human evolution. Yet, influenza virologists, immunologists and evolutionary biologists appear to have given little attention to studying the mechanisms underlying these differences.

In older adults, mortality due to seasonal and pandemic influenza largely affects those with underlying high-risk conditions: cardiopulmonary diseases, diabetes and renal disease. In younger adults those with obesity, asthma and pregnancy are affected. In both young and old, these conditions share one feature in common: each is characterized by alterations in innate immunity that in many instances constitute a form of low-grade inflammation known to cardiovascular scientists as "metabolic syndrome."[42-46] Among children who die of influenza, most have known immune disorders. In those with fatal influenza and no recognized disturbance in immune function, it is possible that unrecognized antecedent events have induced cytokine dysregulation and increased their vulnerability to influenza-related complications and death. In all likelihood, all of these individuals are at increased risk because their "innate immune rheostats" have been set at different and more precarious levels, making them more vulnerable to a loss of innate immune homeostasis.[47]

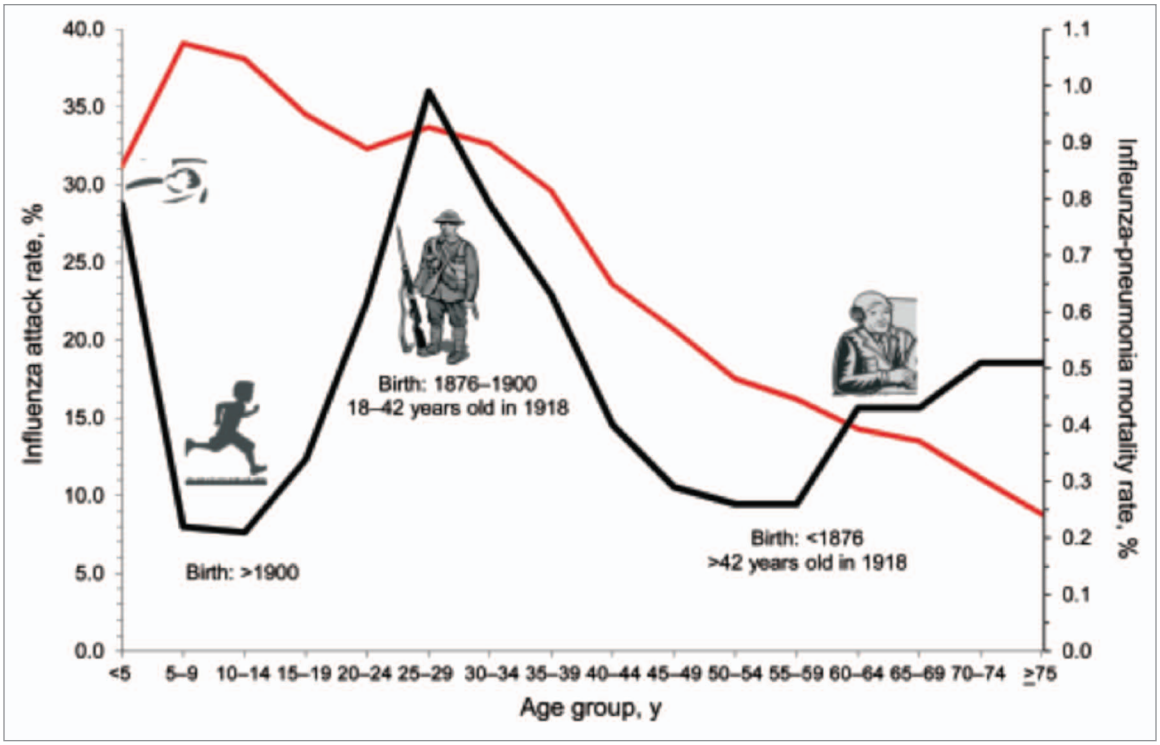

Figure 1. Discrepancy between clinical influenza attack rates and influenza pneumonia mortality rates in the 1918 influenza pandemic (adapted from ref. 38).

The Host Response to Influenza

Human influenza is associated with elevated levels of pro- and anti-inflammatory cytokines and chemokines, and the greater the degree of dysregulation, the greater the likelihood of severe or fatal illness.[48] Even in patients with mild illness, elevated cytokine levels distinguish between those who develop symptoms and those who have asymptomatic infection.[49] Few people with fatal influenza die during the first few days of illness when a pro-inflammatory response dominates. Instead, like patients with sepsis,[50] most die during the second week or later when an anti-inflammatory response and immunosuppression become dominant and virus replication has decreased.[36,40] These changes in the host response have been demonstrated in studies of H5N1 and non-H5N1 influenza viruses in mice,[51] ferrets[52] and non-human primates,[53] and interactions between virus and host factors that determine the course of illness have been discussed extensively by influenza virologists.[54-57]

Many influenza virologists are convinced that virus factors - infecting dose, extent of replication and degree of virulence - principally determine the outcome in influenza, hence their emphasis on controlling the disease with vaccines and antiviral agents.[57-59] No one would argue seriously that these factors are unimportant. Nonetheless, they cannot explain why an inactivated H5N1 virus can cause fatal acute lung injury in mice,[60] nor why survival in the acute lung injury seen in sepsis, pneumonia and influenza is determined by active resolution of inflammation,[61,62] the restoration of pulmonary endothelial barrier integrity,[63] mitochondrial biogenesis[64-66] and changes in energy metabolism.[67,68] Most of all, it is difficult to imagine how factors intrinsic to the virus could have been solely responsible for the different mortality rates seen in children and adults in the 1918 pandemic.[36,40]

A dysregulated host response appears to be the principal factor responsible for fatal influenza. Since timely and affordable supplies of vaccines and antiviral agents won't be available when the next pandemic virus emerges, the challenge to laboratory and clinical investigators is to identify existing agents that can reestablish the host's capacity for self-regulated homeostasis. An abundance of clinical and laboratory research indicates this can be done.

Targeting the Host Response to Pneumonia and Influenza with Immunomodulatory Agents

A growing body of evidence suggests it should be possible to modify the dysregulated host response of patients with community-acquired pneumonia and influenza and improve their survival.[36] For many years, physicians have used 3-hydroxymethyl-3-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), peroxisome proliferator activator receptor (PPAR)α and PPARγ agonists (fibrates and glitazones, respectively) and AMP kinase agonists (metformin) to treat the dysregulated host responses of patients with chronic heart diseases and diabetes mellitus. The clinical benefits and safety of these immunomodulatory agents are widely known. In addition to their effectiveness when given as long-term treatment, they have beneficial effects when given acutely; for example, when statins are given to patients within 24 h following hospitalization for acute myocardial infarction,

they significantly reduce hospital mortality.[69] These agents have also been shown to have overlapping anti-inflammatory and immunomodulatory (pleiotropic) activities in mouse models of systemic inflammation, both sterile [e.g., after endotoxin (LPS) treatment] and infection-induced [e.g., cecal ligation and puncture (CLP)] sepsis.[36]

Observational studies in humans have evaluated the effects of statins in patients with pneumonia (there are no studies of fibrates, glitazones or metformin). Most but not all of these studies have shown that outpatients taking statins (almost certainly for cardiovascular reasons) have reduced rates of pneumonia hospitalization and death.[70-75] Three observational studies have documented the effects of inpatient statin treatment on pneumonia mortality. In one study of 1985 patients, continued statin use in the hospital reduced hospital mortality by 27% [adjusted odds ratio (OR) 0.73; 95% confidence interval (CI) 0.47–1.13; p = 0.15].[76] In a second study of 121,254 inpatients, statin treatment reduced hospital mortality in those not admitted to intensive care by 21% (adjusted OR 0.79; 95% CI 0.71–0.87), but it had no effect on mortality in those who required intensive care (adjusted OR 0.93; 95% CI 0.81–1.06).[77] The third study reported the results of a propensity matched case-control study that used a Department of Veterans Affairs administrative database of patients ≥ 65 y of age hospitalized with pneumonia (11,498 cases and 11,498 controls).[78] Inpatient statin treatment was associated with a 32% reduction in 30-d mortality (adjusted OR 0.68; 95% CI 0.59–0.78). In addition, outpatient statins were associated with a 26% reduction in 30-d mortality (adjusted OR 0.74; 95% CI 0.68–0.82). Outpatient and inpatient use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) were also associated with significant reductions in 30-d mortality, but there was no analysis of combination treatment with a statin and either an ACE inhibitor or an ARB.[78]

No reports have been published of randomized controlled trials of statin treatment of patients with pneumonia. However, a single center clinical trial conducted in 100 patients hospitalized with sepsis has shown that atorvastatin (40 mg/day) significantly reduced progression to severe sepsis (4% in treated patients vs. 24% in controls; p = 0.007).[79]

Immunomodulatory Treatment of Pandemic Influenza

In 2004, it was suggested that statins might be useful in reducing mortality from pandemic influenza.[80] This idea was based on the well-established phenotypic benefits of acute statin treatment in patients with acute myocardial infarction, and the possibility that similar benefits might be seen in patients with severe influenza. Over the next few years, several influenza virologists failed to show that statins could reduce influenza mortality in mice, although none of their studies has been published (DS Fedson, unpublished observations).

Two recent studies failed to show that statins reduce mortality in mouse models of influenza. In one report, rosuvastatin (administered in the diet) failed to protect C57Bl/6 mice infected with H3N2 and WSN influenza viruses, but the infecting doses of virus were very high (LD_{100}) and there was clear evidence that after one or two days the mice stopped eating, and therefore were no longer being treated.[81] In a much larger study, several different statins were tested against several different influenza viruses in BALB/c mice.[82] No meaningful evidence of protection was shown, but again the infecting dose of virus was highly lethal. Moreover, treatment was given for only a few days, and it is well known that early cessation of statin treatment during an inflammatory illness in both mice and humans leads to a rebound hypercytokinemia and increased mortality.[83]

A limited number of laboratory studies have shown the effectiveness of other immunomodulatory agents in mouse models of influenza. Post-infection treatment with resveratrol (a plant polyphenol with immunomodulatory activities)[84] and gemfibrozil[85] significantly improved survival in influenza virus-infected mice, and similar improvements have been demonstrated for pre-infection treatment with pioglitazone[86] and pioglitazone combined with AICAR, a metformin-like drug.[87] In two studies that evaluated the effects of treatment on virus replication, pulmonary virus levels were either unchanged[86] or reduced.[84] A more recent study has shown that treatment of mice with the PPARγ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), starting one day after infection, improved survival from 14% to 79% and markedly reduced pulmonary virus titers.[88] Surprisingly, 15d-PGJ_2 treatment started on day 0 was not protective. Moreover, although protection by 15d-PGJ_2 could be reversed by a specific PPARγ antagonist, treatment with rosiglitazone (a clinical PPARγ agonist that also has non PPARγ activities) on day 0 or day 1 was not protective. In another study, a highly active glutathione derivative (glutathione is an important intracellular antioxidant) strongly inhibited PR8 influenza virus replication in vitro by blocking cytoplasmic maturation of the virus hemagglutinin, and treatment of influenza virus-infected mice reduced mortality 4-fold.[89] Statins, glitazones, fibrates and metformin all upregulate glutathione activity.[90] It is important to note that none of these experimental studies included co-treatment with a recognized antiviral agent.

Reports on the effects of immunomodulatory agents in human influenza are limited to statins. Two reports have appeared on the effects of statins on laboratory-confirmed human influenza. In an observational study of 1520 patients hospitalized in 2009 with pH1N1, preadmission statins were associated with a statistically nonsignificant 28% reduction in hospital mortality (adjusted OR 0.72; 95% CI 0.38–1.33).[91] Unfortunately, the investigators gathered no data on inpatient statin use. More important, an observational study has reported on statin treatment of 3043 older adults hospitalized in 2007–2008 with laboratory-confirmed seasonal influenza.[92] Statins were begun as outpatient treatment in 96% of patients and were either continued or started after hospital admission in 87%. Statin use was associated with a statistically significant 41% reduction in mortality within 30 d of a positive test for influenza virus (adjusted OR 0.59; 95% CI 0.29–0.92; deaths occurred either in the hospital or shortly after discharge). The results of this pivotal study provide compelling evidence to support the concept that immunomodulatory treatment of influenza should work.

Table 1. Cell signaling pathways that might be targeted by immunomodulatory treatment*

• Upregulate HO-1† and decrease TLR signaling by PAMPs and DAMPs
• Downregulate NF-kappaB and pro-inflammatory cytokines (e.g., TNFα, IL-1, IL-6)
• Upregulate anti-inflammatory cytokines (IL-10, TGFβ)
• Upregulate pro-resolution factors (lipoxin A4, resolvin E1)
• Downregulate HMGB1/RAGE and late mediators of inflammation
• Upregulate adipokines (adiponectin) that decrease inflammation
• Upregulate eNOS, downregulate iNOS, restore iNOS/eNOS balance and stabilize cardiovascular function
• Decrease formation of reactive oxygen species and reduce oxidative stress
• Decrease tissue factor and its associated pro-thrombotic state
• Attenuate the C5a-C5aR-related increase in vascular endothelial permeability
• Stabilize the actin cytoskeleton and adherens and tight junctions in endothelial cells, increase pulmonary barrier integrity and decrease vascular leak
• Attenuate acute disease-associated pulmonary hypertension
• Restore the balance between Th17 and Treg cells
• Differentially modify caspase activation and apoptosis in epithelial and endothelial cells, macrophages, neutrophils and lymphocytes in the lung and other organs
• Upregulate AMPK and PGC-1α, improve mitochondrial function and restore mitochondrial biogenesis and metabolic homeostasis

*Adapted from references 36 and 96 and DS Fedson, unpublished observations. †HO-1, heme oxygenase -1; TLR, Toll-like receptor; PAMP, pathogen-associated molecular pattern; DAMP, damage associated molecular pattern; NF-kappaB, nuclear factor kappaB; TNFα, tumor necrosis factor α; IL-1, Interleukin-1; TGFβ, transforming growth factor β; HMGB1, high molecular group box-1; RAGE, receptor for advanced glycation end products; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; C5aR, C5a receptor; Treg, T regulatory; AMPK, adenosine monophosphate-activated protein kinase; PGC-1α, peroxisome-proliferator-activated receptor (PPAR)γ coactivator-1α.

Questions about the Effectiveness of Statins in Treating Influenza

The results of this pivotal study have been questioned because it is thought that patients who received statins were "healthy users."[93] The same reason has been used to claim that observational studies showing the effectiveness of influenza vaccination in reducing hospitalizations and deaths are similarly biased; in other words, vaccination appears to be effective (but is not) because relatively healthy older adults take better care of their health (and get more vaccines) than those who are less healthy, and thus they are more likely not to be hospitalized or die because they are healthier, not because they have been vaccinated.[94] The statins investigators responded to this criticism by listing the steps they took in their analysis to control for healthy user bias.[95] The critics failed to mention that the healthy user bias had already been accounted for by the investigators in their adjusted analysis: the 41% reduction in mortality with statin treatment was in addition to any reduction that might have been attributable to previous influenza vaccination and antiviral treatment.[92]

The results of most observational studies demonstrate the phenotypic effects of statin treatment in reducing pneumonia and influenza mortality. To date, no such studies have been reported on the effects of glitazones, fibrates or metformin, although observational studies of large groups of diabetic patients would be informative. Nonetheless, the known immunomodulatory effects of these agents in other conditions characterized by cytokine dysregulation (e.g., cardiovascular disease, metabolic syndrome, diabetes) as well as their effects in several experimental models of infection and inflammation have provided insights into some of their potential mechanisms of action (**Table 1**; refs. 36, 96, 97 and DS Fedson, unpublished data). Other immunomodulatory agents have been suggested as candidates for influenza treatment.[98] ACE inhibitors and ARBs are among the most promising agents,[78] but there are no studies of their use in experimental models of influenza. Among other agents that are licensed, (e.g., macrolides, cyclooxygenase-2 inhibitors), few data support their use. For other candidate agents (e.g., anti-TNF therapy, mesenchymal stem cells, angiopoeitin-1, high mobility group box-1 antagonists), limited supplies, high costs and/or their investigational status mean that many years will pass before any of them can be considered seriously for clinical trials in influenza patients.

We already have an indication that immunomodulatory treatment might reduce the higher influenza mortality rates of younger adults. In an experiment published in 2008, "children" and "young adult" mice were subjected to ischemia reperfusion injury of the liver.[99] (In "young adult" mice more so than in "children," this condition is highly inflammatory and often fatal). In this study, pre-treatment with rosiglitazone was able to "roll back" the harmful inflammatory response of young adults to the more benign response of children. This important experiment could have implications for patient care in an influenza pandemic. In a study comparing the effects of pH1N1 virus infection in newly weaned and adult ferrets, the immunological and pathological findings in newly weaned ferrets were less severe and the clinical illness was much milder.[100]

The four groups of the immunomodulatory agents mentioned above are now produced as inexpensive generics in developing countries. If these agents could be shown convincingly to reduce mortality in patients with severe influenza, they would be

available to treat patients in any country with a basic health care system on the first pandemic day. For each patient, the cost of this "bottom up" approach would be less than one dollar.[36]

Corticosteroid Treatment of Influenza: A Cautionary Note

Physicians often use corticosteroids to treat patients with sepsis, severe acute lung injury and acute respiratory distress syndrome in the hope that the anti-inflammatory effects of these agents will improve survival. Unfortunately, the evidence supporting their use is weak.[101,102] This includes observational studies in 6650 patients and ten randomized controlled trials involving 1090 patients hospitalized with pneumonia due to pandemic H1N1 virus infection.[102] Some of these studies have even shown that corticosteroids were harmful,[103,104] leading to a spirited discussion of the pros and cons of steroid treatment for viral pneumonia.[105,106]

A full discussion of corticosteroid treatment lies outside the bounds of this review. Nonetheless, it is worth noting the considerable overlap in their cell-signaling pathways and those for the immunomodulatory agents under discussion here (**Table 1** and ref. 106). There is also considerable molecular crosstalk between PPAR agonists and the glucocorticoid receptor.[107,108] Thus, despite encouraging results from the observational studies reviewed above, these similarities argue for caution regarding benefits that might be anticipated from treating influenza patients with statins and these other agents. That being said, fibrates and statins enhance the signaling effects of corticosteroids,[108,109] so combination treatment that includes a corticosteroid might be more beneficial than single agent treatment. In addition, a direct comparison of dexamethasone and pioglitazone treatment of smoke-exposed mice infected with H1N1 influenza A virus showed greater efficacy for pioglitazone.[110]

A Research Agenda for Immunomodulatory Treatment of Influenza Patients

Several years ago, a five-point research agenda was proposed for identifying one or more immunomodulatory agents that might be used to manage patients with pandemic influenza (**Table 2** and ref. 36). If immunomodulatory agents could be shown to be effective, they would be used primarily to treat pandemic patients with severe, life-threatening illness, although for special groups (e.g., health care workers or very high-risk patients) they might also be used for prophylaxis, especially when vaccines and antiviral agents are unavailable.

Since this agenda was first presented, there has been progress on several fronts. We now have good international information on the companies that produce statins, glitazones, fibrates and metformin. We also have information on quantities produced each year, distribution channels and wholesale prices for branded and generic products. For example, a few years ago it was estimated that in 2012, 48 billion doses of statins would be distributed throughout the world (DS Fedson, unpublished observation). Of these doses, 77% would be produced as generics, and the average price per generic dose would be $0.17. Almost 20 billion doses would be distributed in countries outside the United States, Canada and Western and Central Europe. If it were assumed that in a pandemic, 5% (350 million) of the world's 7.0 billion people would need to be treated for ten days (a deliberately exaggerated assumption), 3.5 billion doses would be required. This would account for approximately 7% of the annual consumption of statins worldwide. Information on statins and the other immunomodulatory agents mentioned above needs to be updated. Nonetheless, it is already evident that these drugs are currently available as generics wherever there are physicians who treat patients with cardiovascular diseases and diabetes. In most countries, expensive programs for stockpiling them would not be needed.

Soon after the H1N1 pandemic virus emerged in 2009, several groups of intensive care specialists tried unsuccessfully to initiate randomized controlled trials of statins in pH1N1-infected, ICU-admitted patients.[111,112] The focus on statins was based largely on encouraging findings from observational studies of statins use in patients with sepsis and pneumonia (no such information was available for the other agents). Nonetheless, there is broad agreement that randomized controlled trials will be needed to determine whether immunomodulatory treatments are efficacious. In anticipation of the next pandemic, clinical trials should be organized beforehand so they can be started immediately after the emergence of a new pandemic virus. In the meantime, similar trials conducted in patients with seasonal influenza should be undertaken. Investigators will have to decide whether the trials should be restricted to ICU-admitted patients, who might not benefit,[76,77,113] or include all hospitalized patients at risk of rapidly developing more serious illness.[79] Regardless of their design, the trials will be expensive, so animal studies comparing different immunomodulatory agents will be needed to guide the choice of which agent(s) to evaluate in clinical trials.

Animal Studies of Immunomodulatory Treatment of Influenza

Investigators will need to proceed with caution because the results of laboratory studies might be difficult to interpret.[81,82] For example, studies by several virologists have yet to show that statins are effective in mouse models of influenza, yet many human studies suggest that they are (see above). There is no ready explanation for these discordant results, but it is worth noting that although the molecular mechanisms for the inflammatory responses of humans and mice are in many ways similar, they are quantitatively very different. For example, a comparison of the response of human and mouse macrophages to LPS-induced inflammation showed that the human response was 10,000 times more sensitive to LPS than that of mice.[114]

In mouse models of immunomodulatory treatment, choosing a test virus that more clearly mimics human influenza virus infection could be important (**Table 2**). For example, the mouse-adapted PR8 virus is highly lethal for mice, but markedly less so for man, so a pH1N1 virus might be a better choice. Likewise, choosing an appropriate infecting dose is also

Table 2. Research to identify immunomodulatory agents that might be used to treat pandemic influenza patients*

• Test candidate agents in mice, ferrets and non-human primates to identify agents that might be used to manage patients
• Later study these agents in cell culture and animals to identify molecular mechanisms that explain their beneficial effects
• Document where these agents are produced as generics and determine quantities produced, surge capacities, patterns of distribution and costs to public programs
• Establish a process for managing their global stockpiling before a pandemic or distribution once a pandemic begins
• Plan randomized controlled trials of promising agents to begin immediately upon the emergence of a new pandemic virus

*Adapted from reference 36.

probably important; an illness caused by a dose that is 100% lethal in mice will probably not reflect the spectrum of human influenza because not all patients with severe illness die. The choice of mouse strain might also be critical. Influenza virologists usually use either inbred BALB/c or C57Bl/6 mice,[115] and these two strains have been used in all experimental studies of immunomodulatory agents.[84-89] These strains might not be optimal for determining which agent might best counteract the more intense inflammatory response in man. For example, in a study of host factors involved in the pathogenesis of pH1N1 virus influenza, BALB/c mice, which have a Th-2 bias, were shown to be less suitable than C57Bl/6 mice, which have a Th-1 bias.[116] Neither strain might be as suitable as DBA/2J mice, which have a more intense inflammatory response to influenza virus infection.[117-119] Investigators should also consider testing immunomodulatory agents in mice that have the same high-risk conditions as humans; e.g., pregnancy,[62] obesity[120] and cardiovascular disease.[121] Once the most promising immunomodulatory agent (or combination of agents) has been identified, it should then be studied in ferrets and, if necessary, in non-human primates. In all of these studies it will be important to compare responses in "children" and "adults."

The Broader Implications of Immunomodulatory Treatment for Global Health

Despite compelling arguments for undertaking the laboratory and clinical research needed to show definitively whether immunomodulatory agents would improve survival in severe influenza, virologists and public health officials, including those at the World Health Organization, remain focused on targeting the virus. Yet success with treating the host response to influenza might be extended to the management of several other diseases in which cytokine dysregulation and the loss of homeostatic defense mechanisms leads to poor outcomes; for example, pneumococcal pneumonia,[122] severe malaria,[123] dengue hemorrhagic fever[124] and critical illness associated with trauma[125,126] and burn injury.[127,128]

Almost a half-century ago, physicians and public health officials learned that syndromic treatment of the host response to severe acute diarrheal illness could be accomplished with an inexpensive and universally available oral rehydration solution (ORS).[129] Although vaccines that target a few of the pathogens responsible for diarrheal disease have been developed since then (e.g., cholera and rotavirus vaccines), it is syndromic treatment with ORS that has saved millions of lives. Had decisions been made long ago to ignore the possibility of simple and inexpensive treatment and instead focus only on developing vaccines, these millions would have died. Scientists and health officials responsible for developing a practical response to a global influenza pandemic should learn from this history.

Conclusion

The dysregulated host response seen in severe influenza (and many other conditions) might be treatable with safe, inexpensive generic immunomodulatory agents. Whether these agents will actually be effective in routine clinical care needs to be demonstrated in further laboratory and clinical research. Nonetheless, it should be clear to everyone that such treatment would be of immense practical importance to global public health. Until now, influenza virologists have been reluctant to undertake experiments to identify potentially useful and widely available agents that investigators could test in clinical trials and physicians could use to manage their patients. Until they do, public health officials will have no alternative but to recommend that most of the world's people confront the next global influenza pandemic with little more than hand washing and social distancing. These "technologies" represent the best of 19th Century public health practice. In the 21st Century, we can and should do much better.[36,130]

The debate about H5N1 transmissibility research should be about more than how to define its boundaries, important though this may be. The controversy presents influenza virologists, biosecurity experts and public health officials with a new opportunity to jointly define a research agenda to identify existing immunomodulatory agents that could be used in a practical response to a global influenza pandemic. This opportunity must not be wasted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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